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B. T. GALLOWAY, *Chief of Bureau.*

THE DESTRUCTION OF CELLULOSE BY BACTERIA AND FILAMENTOUS FUNGI.

BY

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266
2

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., September 12, 1912.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 266 of the series of this Bureau a manuscript entitled "The Destruction of Cellulose by Bacteria and Filamentous Fungi." This paper was prepared by Messrs. I. G. McBeth, Physiologist, and F. M. Scales, Assistant Soil Mycologist, of the Office of Soil-Bacteriology and Plant-Nutrition Investigations, and has been submitted by the Physiologist in Charge with a view to publication.

New species of cellulose-destroying organisms are described, as well as special methods and new culture media adapted to their isolation and identification.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

ILLUSTRATIONS.

PREFATORY NOTE.

Of the phenomena relating to the art of argiculture none is more interesting or more vitally important than the formation of starch, sugar, cellulose, and similar compounds by green plants. The action of chlorophyll, as yet unexplained, by which plants utilize the energy of the sunlight to synthesize carbon dioxid and water into carbohydrates is logically the most fundamental question of plant physiology, for it is the enormous quantity of potential energy thus accumulated that directly or indirectly makes possible the continuance of all vital processes. Not only animal life which, generally speaking, is dependent upon plants for food supply, but even the successful growth of crop plants is largely controlled by the decomposing carbonaceous material in the soil.

Both in the Eastern and Western States the natural maintenance of the supply of available nitrogen is seldom considered when determining the most desirable system of farm management in any region, yet, as scientific research and experience in the field agree in showing, the soil itself may fix and render available to the crops considerable quantities of nitrogen, which is the highest priced of the plant foods if it be purchased as commercial fertilizer. Furthermore, as far as our experience extends, all of the fixation of atmospheric nitrogen in the soil is dependent upon the growth of microorganisms which must have large quantities of soluble carbon compounds for food. As by far the larger part of the carbonaceous material added to the soil as dried roots, stubble, green manure, etc., is cellulose, a substance which is unusually refractory and can not be used as such for food by nitrogen-fixing organisms, the biological phenomena which transform the cellulose to soluble compounds are obviously important. It is not only as a possible food supply for nitrogen-fixing organisms, however, that a soil requires a constantly replenished supply of cellulose. Its decomposition under proper soil conditions and in association with the decay of nitrogenous compounds makes possible the formation of the so-called soil humus. The beneficial effects of the presence of indefinite humic compounds upon the physical character and fertility of a soil are generally recognized throughout the agricultural regions of the United States.

The gradual processes of decay that are depended upon to maintain many of the factors of a soil's fertility are probably as complex as the microflora and fauna of the living soil itself, and it is chiefly through the discovery and comprehension of the essential biological phenomena relating to the growth of plants that permanent improvement in crop production can be made possible. The decomposition of cellulose is apparently one of the fundamental questions of the decay of organic material, and though a subject of research in foreign countries for many years, it has been but imperfectly understood. The investigations, therefore, of which this bulletin is a progress report, are regarded as of unusual importance.

KARL F. KELLERMAN,
Physiologist in Charge.

OFFICE OF SOIL-BACTERIOLOGY INVESTIGATIONS,
Washington, D. C., September 10, 1912.

266

B. P. I.—779.

THE DESTRUCTION OF CELLULOSE BY BACTERIA AND FILAMENTOUS FUNGI.

INTRODUCTION.

The important functions of fission fungi is to dissolve and again place in circulation the complex organic substances which have ceased to live. Without their activity the cycle of change to which all organic matter is subject would come to a standstill and the food supply of plants would soon be depleted. It is well known that through the agency of micro-organisms all vegetable matter is gradually transformed into the complex mixtures ordinarily known as humus and that we are at least partially dependent upon the quantity and quality of the humus compounds for the fertility of the soil.

It is true that numerous chemical researches have added materially to our knowledge of these organic or humic compounds, but since the biological processes involved are the dominant factors in determining the manner in which complex organic substances are split up, a systematic study of the organisms which bring about the decomposition of vegetable matter and the formation of plant food is imperative.

Vegetable substances may be roughly divided into two great classes, nitrogenous and nonnitrogenous. In the decomposition of nitrogenous matter we are concerned chiefly with the fate of the nitrogen, a part of which seems to be invariably returned to the atmosphere. It is well known that this loss may be considerable. Fortunately, however, nature has provided a means of restoring this lost nitrogen through the activity of certain so-called nitrogen-fixing micro-organisms. A study of these organisms and the conditions under which they are able to fix nitrogen has shown that the process is controlled in large measure by the available supply of organic carbon.

On examining plant tissues we find a large percentage of the carbon content locked up in the celluloses; these are inert compounds which resist the attacks of the ordinary putrefactive bacteria and until broken down into simpler compounds are inaccessible to nitrogen-fixing bacteria. Little is known of the biological processes involved in the destruction of cellulose. It is true that many foreign investigators have studied cellulose ferments, but generally the work has

been done in a tentative way, and no suitable methods for isolating these essential organisms have been worked out. Consequently, our ideas of the number and nature of the cellulose ferments have been very inadequate. Investigators have also devoted much attention to the products resulting from the fermentation of cellulose, but they apparently have been working with impure cultures and their conclusions are of doubtful value.

Believing that little progress could be made in the study of cellulose decomposition until satisfactory methods for isolating these organisms had been perfected, thus giving opportunity to learn something of their cultural characteristics, we have endeavored to work out methods to isolate and study them. The purpose of this bulletin is to review briefly the work of earlier investigators, point out the inadequancy of our present knowledge of cellulose fermentation, and set forth the results obtained from our own studies in the hope that they may be of value to other investigators.

HISTORICAL REVIEW OF INVESTIGATIONS OF THE DESTRUCTION OF CELLULOSE.[1]

BACTERIOLOGICAL AND CHEMICAL INVESTIGATIONS.

The fermentation of cellulose was first attributed to the activity of microorganisms by E. Mitscherlich in 1850. He noted that when slices of potato were immersed in water and held in a warm place the cellulose, which constitutes the main portion of the cell walls, was destroyed. First, the cells became separated from each other, and soon afterward the walls were broken down and the starchy material fell out. By filtering the solution and dropping in fresh potato the fermenting process was greatly accelerated. Microscopic examinations showed no trace of a mold growth, but Mitscherlich observed swarms of vibrios, which he believed to be the active agents of cellulose fermentation.

Four years later Haubner showed that it was impossible to recover from the feces more than 50 per cent of the crude fiber fed to ruminants. He obtained similar results with wood shavings which had been treated with acid and alkali, and also with thoroughly washed paper when fed with hay and bran to sheep. Haubner's work was soon confirmed by Henneberg and Stohmann. Through the experiments of Hofmeister, Zuntz, Knierem, Weiske, Lehmann, and others similar results were obtained with horses, sheep, goats, rabbits, etc. Although undertaken primarily to determine the nutritive value of crude fiber in foodstuffs, these investigations no doubt did much to stimulate later investigation which sought to determine the causative agent of cellulose fermentation.

[1] For bibliography, see pages 47 to 50.

In 1865 Trecul undertook a study of microorganisms in macerated plant tissues. He observed and described three forms, which he placed in a distinct genus, Amylobacter, and divided into three subgenera. This generic name was selected because these organisms stained blue with iodin. He believed that starch or cellulose favored the production of these bodies.

For our early knowledge of cellulose fermentation we are much indebted to the work of Popoff, who in 1875 first pointed out the connection between cellulose fermentation and the formation of methane. Methane had long been known to exist in sewers and marshes and had been found in fermenting horse manure by Reiset as early as 1856; however, no successful attempt had been made to determine the source of the gas. For experimental material Popoff used slime from the sewers of Strassburg. The material was mixed with sufficient water to make a thick solution, poured into large flasks, and preparations made to collect the gas over quicksilver. An analysis of the gas showed that considerable quantities of methane mixed with other gases had been produced. The optimum temperature for the gas formation was found to be 38° C. to 40° C.; at 45° C. the activity was much weakened, and at 50° C. it came to a standstill; lower temperatures were also shown to be very unfavorable. Popoff further showed that the fermentation process could be altered at will by the addition of antiseptics. The next step was to show that the formation of methane could result from the destruction of pure cellulose. With this end in view a quantity of pure Swedish filter paper was immersed in water and inoculated with a small quantity of slime known to contain the methane ferment. The paper was destroyed and a large quantity of gas was formed, which on examination proved to be a mixture of carbon dioxid, methane, hydrogen, and nitrogen. The gases collected during the first two weeks and again several weeks later were analyzed with the result shown in Table I.

TABLE I.—*Analyses of mixed gases formed by the decomposition of Swedish filter paper.*

Gases found in the mixture.	Collected at the end of—	
	Two weeks.	Several weeks.
	Per cent.	*Per cent.*
Carbon dioxid	25.70	34.07
Methane	14.42	37.12
Hydrogen	14.36	1.06
Nitrogen	45.52	27.75

It appears, therefore, that the quantity of hydrogen decreased with the duration of the experiment, while at the same time there was an

increase in the formation of methane, so that in the end the quantities of carbon dioxid and the methane were about equal. In studying other substances Popoff found that a methane fermentation could be produced from gum arabic as well as from cellulose. He is of the opinion that the typical cellulose ferment gives rise to carbon dioxid and methane only, and that the presence of hydrogen in the gas is due to other fermentation processes.

In 1877 Van Tieghem, in working out the life history of the amylobacter of Trecul, found that it was motile, as Nylander had done twelve years before, and classified it as a bacillus. He further found that it was an anaerobic, cellulose-dissolving organism and that it grew readily in soluble starch and cellulose, first reducing them to dextrin and then converting the dextrin into glucose, which was fermented with the production of carbon dioxid, hydrogen, and an acid which inhibited the growth of the organism unless neutralized with calcium carbonate. No cytase was liberated in solution and the cellulose was dissolved only when in direct contact with the organism. He proved the cellulose-dissolving power of the organism to his own satisfaction by inoculation experiments in solutions containing macerated radish. However, he found that the organism did not act the same on all plant tissues; in a word, that *Bacillus amylobacter* could not attack all celluloses.

Results of studies on fermentation processes were published by Prazmowski in 1880. He described two species to which he attributed cellulose-fermenting properties and to which he gave the names *Clostridium polymyxa* and *Vibrio rugula*. The former was found to have only a weak fermentive power in dextrin solutions, but was extremely active in preparations of cooked potato and lupine seed; its activity on starch and cellulose is described as very vigorous. An analysis of the gas formed showed only hydrogen and carbon dioxid.

Vibrio rugula is of especial interest because the description given is so similar to that given later by Omelianski for his so-called hydrogen and methane ferments. In young cultures the rods were unusually thin, about 8 microns long, and showed a characteristic curved structure which made it easy to separate them from other species; later the rods became uniformly thicker, the end swelled up, and a round spore appeared. The young rods were actively motile and the organism was classed as an anaerobe. In infusions of plant tissue the organism was found to surround the cell walls, which were soon dissolved. Prazmowski also made a study of an organism which stained blue with iodin; to this he gave the name *Clostridium butyricum*, although he regarded it as synonymous with *Vibrion butyrique* Pasteur, *Amylobacter* Trecul, *Bacillus amylobacter* Van Tieghem, and *Bacterium navicula* Reinke and Berthold.

In an extensive series of experiments inaugurated in 1880 Tappeiner has given us interesting data on the disappearance of cellulose in the digestive tract of herbivorous animals. Incidentally he made a study of the compounds produced by cellulose fermentation. Pure cellulose in the form of cotton and filter paper was placed in flasks containing a rich nitrogenous solution. In one series of experiments a 1 per cent neutral flesh extract was poured into flasks; pure cellulose in the form of cotton was added, sterilized, and inoculated with a drop of material from the stomach. It was observed that the flesh-extract solution invariably resulted in a fermentation independent of the typical cellulose ferment; therefore, a check flask containing only the flesh extract was held under the same conditions as the flesh-extract cellulose flask. The result of one such experiment is shown in Table II.

TABLE II.—*Measurement of gas formed by the decomposition of cellulose and flesh extract and of flesh extract alone.*

Gas formed.	Flask 1, cellulose and flesh extract.	Flask 2, flesh extract (check).
	C. c.	*C. c.*
Carbon dioxid	191.00	10.10
Hydrogen	1.70	3.00
Nitrogen	10.40	8.60
Methane	88.30	4.20

The fatty acids found in flask 1 amounted to 1.6651 grams and consisted of 2.2 parts acetic acid to 1 part butyric acid, giving a carbon content of 0.7414 gram. The fatty acids found in flask 2 amounted to 1.005 grams and consisted of 2.1 parts acetic acid to 1 part butyric acid, giving a carbon content of 0.4918 gram. Subtracting the products found in flask 2 from those in flask 1 we have the products formed in the decomposition of cellulose, with a carbon content as follows: Carbon dioxid, 0.0966 gram; methane, 0.0436 gram; acids, 0.2496 gram. Total, 0.3898 gram.

In the fermentation of the cellulose 0.4165 gram of carbon was lost, being slightly more than the quantity recovered in the by-products.

A separate series of experiments was conducted with Nageli's solution [1] and 0.6 per cent asparagin. Flasks of 360 cubic-centimeter capacity were filled with this solution and 3.5107 grams of dry cotton added. The fermentation resulted in the formation of carbon dioxid, hydrogen, and nitrogen. Fatty acids, including acetic, propionic, and butyric, were also produced. In the control flask no gas was formed and only traces of fatty acids. It is noted in this experiment that

[1] Potassium phosphate (dibasic), 0.20 gram; magnesium sulphate, 0.04 gram; calcium chlorid, 0.02 gram; water, 100.00 c. c.

0.9447 gram of cellulose was destroyed without any methane formation, while in the previous experiment a considerable quantity of methane was produced even in the flesh-extract flask to which no cellulose was added.

The interesting series of experiments commenced by Hoppe-Seyler in 1881 did much to confirm the growing belief that cellulose is decomposed by a methane ferment. The main experiment is as follows: On December 2, 1881, 25.773 grams of pure filter paper were added to a 1,101 cubic-centimeter flask inoculated with a small quantity of slime and sufficient water added to bring the mixture up to about 700 cubic centimeters. The flask was protected from the light by a double layer of black paper and preparations were made to collect the gas over quicksilver. The flask was kept in this condition four years. In the early part of the experiment the gas formation was very active, toward the end of the year 1883 it became weaker, and in the second half of the year 1885 was scarcely apparent. The experiment was discontinued on December 6, 1885. Gas analyses to the number of 95 were made from time to time, the summary of which showed 3,281 cubic centimeters of carbon dioxid and 2,571 cubic centimeters of methane. An examination of the contents of the flask at the end of the experiment showed that 15 grams of cellulose had been consumed, and as no other appreciable quantity of by-products could be found the author concluded that cellulose is dissolved according to the following formula:

(1) The hydration of the cellulose with the formation of a hexose,

$$C_6H_{10}O_5+H_2O=C_6H_{12}O_6;$$ and

(2) the destruction of the carbohydrate with the formation of equal quantities of carbon dioxid and methane,

$$C_6H_{12}O_6=3CO_2+3CH_4$$

Hoppe-Seyler says in one part of his work that the formation of carbon dioxid took place only when he found in his solutions living bacteria which showed no difference from *Bacillus amylobacter* of Van Tieghem, and he is therefore of the opinion that the destruction of cellulose was due to the activity of this organism.

Gayon in 1883 and 1884 noted the presence of methane in fermenting manure, and from 1 cubic meter of this material well moistened with water and held at 35° C. he succeeded in obtaining as much as 100 liters of the gas in 24 hours. This fermentation he attributed to an extremely small anaerobic organism which was cultivable in nutrient solutions containing either straw or paper, in which it attacked the cellulose and liberated carbon dioxid and methane.

The extensive experiments of Deherain in 1883 and 1884 on the aerobic and anaerobic fermentation of straw and manure showed that in a pile of manure under natural conditions the gas liberated

at the bottom of the pile is pure methane and carbon dioxid, while no methane is produced near the surface unless the manure is wet, when as much as 10 per cent of the gas produced may be methane. In hermetically sealed flasks the fermentation soon stopped, but on opening the flasks and resealing them the fermentation began anew, though it continued for but a short time. He concluded from this result that the methane ferment is not a strict anaerobe. Occasionally a fermentation produced hydrogen and carbon dioxid and gave a slightly acid reaction, due to the formation of butyric acid, while the pure methane fermentation was always neutral. A microscopic examination of the liquid of two such solutions showed in both cases numerous extremely fine rods, which were almost identical, and the butyric ferment in addition where hydrogen was produced. Deherain tried Pasteur's method of successive cultures, but did not reach an absolute conviction concerning the differences between the two organisms. A few drops of a manure infusion inoculated into dextrin and cane sugar solutions gave hydrogen, while a similar inoculum in a solution containing paper gave methane. A later experiment under the same conditions reversed these results in that 9 per cent of gas from the fermentation of cane sugar was found to be methane, while hydrogen was secured from the decomposition of paper. This evidence was thought sufficient to show the presence of two different anaerobic ferments, one hydrogen and the other methane. It sometimes happened in experiments with straw that an acid fermentation took place and that the dominating gas was methane. This production of acid he believed to be due to a fermentation of sugar, producing hydrogen and butyric acid, and that such fermentation was succeeded by the regular methane fermentation.

The results of a study of the fermentation of manure by Schloessing in 1889 showed that the anaerobic fermentation was much more active at 52° than at 42° C., and that methane was the predominating, if not the only, combustible gas given off. Three years later he and his son carried on some experiments to determine what part bacteria play in the aerobic and anaerobic fermentation of manure at different temperatures. The aerobic work showed that no combustible gas was produced under these conditions and that the bacteria were very active up to 72.5° C., but that at 81° C. all action ceased; in an atmosphere of carbon dioxid he obtained a methane fermentation at 52° but none at 66° C. Once he obtained methane from cow manure and hydrogen from horse manure at 58° C. In one experiment 124.4 grams of fresh horse manure containing 76 per cent moisture were kept in an atmosphere of carbon dioxid for two months at 52° C., and in that time generated 4,217.5 cubic centimeters of carbon dioxid and 4,577.4 cubic centimeters of methane,

which are equivalent to 4.72 grams of carbon. In the first 500 cubic centimeters of gas produced 15.8 cubic centimeters of hydrogen were also found. An analysis was made to determine the quantity of each element in the dry material of 124.4 grams of manure. The results are shown in Table III.

TABLE III.—*Analysis of 124.4 grams of horse manure (dry).*

Stage.	Carbon.	Hydrogen.	Oxygen.	Nitrogen.	Minerals.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Before fermentation	12.67	1.653	10.70	0.453	3.69
After fermentation	7.92	1.125	7.08	.392	3.79
Loss (−) or gain (+)	−4.75	−.528	−3.62	−.061	+.10

The loss of 4.75 grams of carbon corresponds very closely with the 4.72 grams found in the carbon dioxid and methane obtained during the fermentation and represents 37.5 per cent of the carbon in the fresh manure.

The methane fermentation of cellulose was likened to the alcoholic fermentation of sugars by Berthelot in 1889, in that it is determined by living agents, is accompanied by a fixation of the elements of water, and has a similar thermochemical mechanism. He represented the fermentation as taking place so that all the hydrogen of the water enters into one of the products (methane) while all the oxygen goes to form carbon dioxid. The total heat thus liberated was 41 calories, the products being gaseous.

The effect of alkalinity and aerobic or anaerobic conditions on the progress of fermentation and mode of decomposition of straw was investigated by Hebert in 1892. The importance of alkalinity in this fermentation was tested by adding solutions containing from 5 to 10 per cent of potassium carbonate, ammonium carbonate, and ammonium phosphate to dry powdered straw of known composition. This straw suspension was inoculated with several cubic centimeters of urine and incubated at 55° C. After four days the anaerobic flask containing 5 per cent of salts had produced the greatest quantity of gas, which was composed of equal parts of carbon dioxid and hydrogen, but a week later without any change in conditions this flask began producing methane. The predominance of either of the carbonates made no appreciable difference in the rapidity of fermentation, but an excess of ammonium carbonate gave hydrogen at first and methane six days later and an excess of potassium carbonate gave methane in the beginning. The composition of the straw before and after three months' fermentation is given in Table IV.

TABLE IV.—*Composition of straw before and after fermentation.*

Constituent.	Initial composition of straw.	Composition of straw after 3 months' fermentation.	
		Anaerobic fermentation.	Aerobic fermentation.
	Per cent.	*Per cent.*	*Per cent.*
Water	9.52	.....	.....
Nitrogenous materials	4.87	12.50	14.06
Chlorophyll materials	.93	1.36	.62
Reducing sugars	2.43	.00	.00
Dextrin	.05	.00	.00
Gums, tannins, acids	.60	1.26	1.60
Cellulose	28.25	23.30	25.65
Vasculose	28.03	22.70	28.25
Straw gum (into xylose)	20.00	26.00	19.00
Ash	7.15	12.80	12.80
Total	101.83	99.92	101.98
	Gram.	*Gram.*	*Gram.*
Weight of dry straw	0.4524	0.2645	0.2690
Weight of cellulose in the straw	.1412	.0615	.0689

The results show that in both fermentations the straw lost about half its weight and that this loss occurred principally in the three elements most abundant in straw, namely, cellulose, vasculose, and straw gum. The straw first lost all or part of the substances most easily attacked, as chlorophyll materials, gums, tannins, glucose, and dextrin, the higher carbohydrates, cellulose, and straw gum disappearing afterwards; the loss of the former amounting to as much as 50 per cent (7 or 8 grams). Finally the vasculose was partly dissolved in the solution and partly oxidized to carbon dioxid and water. The organisms appeared to work as vigorously under aerobic as under anaeorbic conditions, and Hebert was unable to decide from his figures what are the most favorable conditions for the destruction of cellulose.

Van Senus in 1890 published an extensive treatise on the decomposition of cellulose. He noted the rapid decomposition of cotton, pieces of bean, potato, etc., when inoculated with slime and held under favorable conditions, and attributed these decomposition processes to the joint activity of two organisms. One of them, *Bacillus amylobacter*, which he describes as a rod-shaped organism, 0.8 μ wide and 2 to 10 μ long, stains blue with iodine and forms spores when air is admitted, which then germinate only when air is excluded He showed that *B. amylobacter* in flesh-extract solution with cellulose (cotton, paper, crude fiber, etc.) under no conditions could ferment the cellulose; with sterilized beans, potatoes, etc., the walls were not broken down but only separated from each other, probably through the formation of the ferment pectase. The other organism, isolated from the intestines of the rabbit, is much smaller and, like *B. amylo-*

bacter, has no power to ferment cellulose in pure culture; however, when these organisms were grown in association a destruction of cellulose was secured. The reason for this result, in the opinion of this author, is the production of harmful products by the fermentation of the cellulose, and the presence of another organism is necessary to render these products less injurious. Van Senus is of the opinion that methane is not formed directly by the fermentation of cellulose and that the destruction of cellulose always results in the formation of hydrogen, carbon dioxid, and acetic acid, the action of the hydrogen upon the acetic acid reducing it to aldehyde, alcohol, and finally to methane.

In 1894 Omelianski began an investigation of bacterial cellulose destruction by inoculating a nutrient solution containing filter paper with slime from the River Neva, and incubating at 30° to 35° C. The paper was soon changed into a yellowish, transparent, gelatinous mass, which later disappeared, leaving only a slight residue. A long, extremely slender bacillus with a round polar spore was present in great numbers. In order to get a pure culture of this cellulose-fermenting organism, Omelianski employed Winogradski's "method of elective culture," selecting for this purpose a nutrient solution almost void of organic nitrogen and incubating the culture anaerobically, as the results obtained by Hoppe-Seyler had showed that these conditions were favorable for bringing about a predominance of the desired organism. After a sufficient number of transfers had been made this slender rod, *Bacillus fermentationis cellulosae*, was almost the only one present in the culture, and when inoculated into solutions containing cotton and cellulose of cabbage, turnip, and pith of the elder tree which had been precipitated from Schweitzer's reagent, it produced a vigorous fermentation with the liberation of hydrogen and carbon dioxid. A little methane generated in the first cultures was believed to be due to another organism which disappeared in transferring. A 300-cubic-centimeter flask was filled with a solution made as follows:

Potassium phosphate	1.0 gram.
Magnesium sulphate	.5 gram.
Ammonium sulphate	1.0 gram.
Sodium chlorid	Trace.
Distilled water	1,000.0 c. c.

To this solution were added 3.4743 grams of dry filter paper and 5.7698 grams of calcium carbonate. It was then inoculated with the cellulose ferment, purified by the elective culture method, and incubated 13 months at 35° C. During this time the volume of carbon dioxid in the total gas increased from 15 per cent at first to 98 per cent, and toward the end of the period dropped to 80 per cent. At

the conclusion of the experiment an analysis of the solution showed the presence of 2.2402 grams of acetic and butyric acids, in the ratio of 1.7 to 1, and of gaseous products consisting of 0.9722 gram carbon dioxid and 0.0138 gram hydrogen, making the total weight of by-products produced 3.2262 grams. A loss in cellulose, amounting to 3.3471 grams, was noted, being somewhat greater than the total weights of the by-products found. Valerianic acid, higher alcohol, products giving the odor of decaying cheese, and dissolved hydrogen were not measured. These unmeasured products might account for the difference of 0.1209 gram between the cellulose added and the products obtained. According to these figures 70 per cent of the cellulose used is converted into fatty acids, while hardly 30 per cent is liberated as gas.

In 1904 Omelianski published his method for the separation of the two cellulose-destroying organisms, one called the "hydrogen bacillus," formerly *Bacillus fermentionis cellulosae*, and the other the "methane bacillus." The vegetative cells of the methane organism appeared to form spores more readily than those of the hydrogen bacillus, so to obtain a pure culture of the latter he first heated the inoculating material to 75° C. for 15 minutes in order to kill all the germinating methane organisms, and after several repetitions of this process his culture was apparently free from this organism. After five or six generations the surface of the paper was covered with a bacillus 4 to 8 μ long and 0.5 μ wide, the rods later reaching a length of 10 to 15 μ without gaining in thickness. They never formed chains and took the ordinary anilin stains readily, but would not color blue with iodin. Slightly curved or irregular spiral forms were abundant when fermentation was going on, and in older cultures the rods had a round polar spore 1.5 μ in diameter. When the paper had been destroyed there were many free spores and few rods in the solution. These spores were found to withstand a heat of 90° C. for 25 minutes, so the cultures were freed of nonspore formers in this way. After such heating there still persisted in his cultures a large bacillus with an oval polar spore, while another contaminating organism with a round polar spore and very much like the true cellulose ferment was occasionally present. The former grew readily on solid media like agar jelly, filter paper soaked in gummy salt solution, carrots, summer cabbages, turnips, and potatoes, either alone or in association with other bacteria, but the true hydrogen ferment did not grow on any of these media except that in one case some very small, yellow, liquefying, semitransparent colonies of the cellulose bacteria appeared on a potato plate; they were scarcely apparent without a magnifying glass, and this medium was evidently not a favorable one, as a heavy inoculation gave only a few colonies.

Omelianski asks, "Was the mother culture not absolutely pure or is the growth an association of bacteria?" Inoculation from these colonies gave fermentation of cellulose in only one case and that soon stopped. However, the organism from these colonies had all the morphological characteristics of the bacteria found in the fermenting paper, and he concludes that it was without doubt a pure culture of this bacillus, although it did not give a satisfactory fermentation.

A test to determine whether it was an associative action with the bacillus forming the oval spore gave negative results. The methane ferment was obtained by inoculating a flask containing the mineral salt solution, paper, and chalk with canal slime or fresh horse manure, and incubating anaerobically at 35° C. A microscopic examination of the paper showed that it was covered with an organism similar to the hydrogen bacillus, but thinner and more delicate in outline. The culture was purified by transferring and heating to kill nonspore formers until it presented a microscopically pure picture, appearing as a rod 5 μ long and 0.4 μ wide, with a round polar spore 1 μ in diameter. Morphologically these organisms might be classed as the same species, but physiologically they were very different, for one produced hydrogen and the other methane.

In later investigations Omelianski points out that methane may be produced not only from cellulose but from acetates, pentoses, pentosans, butyrates, lactates, and protein bodies, which, he believes, indicates that the number of reactions in nature which involve the formation of methane is no smaller, perhaps, than the fermentation processes leading to the evolution of hydrogen.

Experiments showing the destruction of the inner tissue of the turnip due to the parasitism of *Pseudomonas campestris* were reported by Smith in 1902. The leaves of the plant were inoculated with a pure culture of this bacterium. The disease moved downward, and sections of the root 52 days after inoculation showed the bacteria to be very abundant in the inner part, although the root was entirely white and sound externally. Cultures made from the diseased interior yielded only *P. campestris*. Carefully prepared sections showed all stages in the solution of the cell walls, from single cells or vessels occupied by the bacteria to cavities filling the place formerly occupied by hundreds of cells and filled with the bacteria and remnants of the cell walls.

Experiments by Van Iterson in 1904 have shown that the fermentation of cellulose may be caused by aerobic as well as by anaerobic bacteria. According to his results the anaerobic processes fall into two groups:

(1) Without the presence of nitrates the cellulose may undergo a hydrogen or methane fermentation.

(2) In the presence of nitrates the cellulose is destroyed by denitrifying bacteria according to the following formulæ:

$$5C_6H_{10}O_5+24KNO_3=24KHCO_3+12N_2+6CO_2+13H_2O.$$
$$C_6H_{10}O_5+8KNO_2=4KHCO_3+2K_2CO_3+4N_2+3H_2O.$$

The destruction of cellulose under aerobic conditions also falls into two classes:

(1) If the medium is slightly alkaline, certain aerobic bacteria will play the principal rôle.

(2) If the medium is acid, then the molds and higher fungi are the active agents of destruction.

In a simple nutrient solution containing dibasic potassium phosphate, potassium nitrate, and filter paper inoculated with a cubic centimeter of canal water and kept at 35° C., the process started in 6 days, and in about 15 days all of the nitrate added and all of the nitrite formed in the early stages of fermentation had disappeared. Analysis of the gas produced showed only carbon dioxid and nitrogen, with no trace of hydrogen or methane. Thus, the process here would seem to be entirely different from the processes resulting in the production of hydrogen or methane.

For the study of cellulose destruction by aerobic bacteria the following solution was prepared:

Tap water	100.00 c. c.
Paper	2.00 grams.
Ammonium chlorid [1]	.10 gram.
Potassium phosphate (diabasic)	.05 gram.
Calcium carbonate	2.00 grams.

After inoculating with sewer slime the fermentation starts in five to six days, after which it goes on very rapidly. The fermenting paper was found to contain a variety of forms, among which was a very small bacillus frequently associated with a large micrococcus. The former was believed to be the active agent of fermentation and was given the name of *Bacillus ferruginus*. The micrococcus is described as having no cellulose-dissolving power in itself but as stimulating *B. ferruginus* to greater activity when associated with it.

Experiments by Macfadyen and Blaxall with thermophilic bacteria in 1899 showed that these organisms may be very active destroyers of cellulose. A nutrient solution containing pure filter paper was inoculated with a small quantity of soil and incubated at 60° C. The result was an active development of gas and odor, and in 10 to 14 days the filter paper was completely broken up. The experiments were repeated with filter paper and also with films of cellulose hydrate obtained from the solution of cotton fiber in the form of thiocarbonate.

[1] Potassium nitrite, potassium nitrate, peptone, or magnesium ammonium phosphate may replace the ammonium chlorid.

The following results were reported on the solutions which showed a disintegration of cellulose by the thermophilic organisms:

(1) No reduction CuO in original or after boiling with acid.

(2) No other evidence of any proximate products of resolution, i. e., carbohydrates of dimensions NC_6.

(3) On distillation 25 c. c. gave volatile acid=1 c. c. of normal NaOH, containing acetic and butyric acids. Residue gave traces only of furfurol on distillation with HCl (1.06 s. g.).

It appears that the destruction has been, for the most part, complete, probably to CO_2 and CH_4. On further investigation you may be able to get an intermediate stage or an organism acting less severely.

All the above results were brought about by mixtures of thermophilic bacteria occurring naturally in the soil, and the action appeared to be of a symbiotic nature. Their action resulted in a complete disintegration of filter paper, fibrous cellulose, and esparto cellulose.

Distaso in 1911 described an organism isolated from the intestinal flora of the chicken, to which he gave the name *Bacillus cellulosae desagregans*, because it was found to be capable of destroying cellulose. When cultivated in mineral solutions with pure cellulose (Berzelius filter paper) the paper is disintegrated, forming flocci or fibers; action never goes further and this author is not sure whether the filter paper is thoroughly transformed. The organism does not stain by Gram's method; forms oval subterminal spores; is aerobic in nature; grows in sugar gelatin; never gives off gas; grows well at 37° but not at 22° C.; produces no indol; grows only feebly on glucose; does not assimilate lactose, maltose, or saccharose, but transforms starch into glucose rapidly.

In 1911 Choukevitch in a study on the bacterial flora of the large intestine of the horse always obtained a fermentation of cellulose in Omelianski's nutrient solution when inoculated with several loopfuls of the intestinal contents. In the fermenting solutions a small organism (*Bacillus gasogenous*) was always found, which morphologically resembled the hydrogen and methane ferments described by Omelianski. Neither a pure culture of this organism nor any of the others which he isolated from the intestine of the horse was able to ferment cellulose.

The most recent contribution to our knowledge of cellulose destruction is that of Kellerman and McBeth, whose report is a preliminary one of work undertaken by the Office of Soil-Bacteriology Investigations. In this report special attention is given to that portion of Omelianski's work from which he concluded that cellulose undergoes either a hydrogen or methane fermentation. The impurity of Omelianski's cultures are discussed, and three new species of cellulose ferments isolated from his cultures (*Bacillus rossica, B. amylolyticus,* and *Bacterium flavigena*) are described.

INVESTIGATIONS WITH FILAMENTOUS FUNGI.

Early experiments with parasitic fungi indicated that many of these organisms were able to make their way into plant tissues by piercing the cell membrane. Such observations were made by Kühn in his study of blight-producing fungi, by the brothers Tulasne in their study of the rusts, and by De Bary in studies with Peronospora. Some years later Marshall Ward was able to watch the penetration of the cellulose walls of the lily bulb by parasitic fungi. The walls became swollen and evidently somewhat softened, which condition he believed to be due to the production of a ferment drop at the tip of the hyphæ. Miyoshi has recently observed a similar phenomenon with *Penicillium glaucum*, *Botrytis bassiani*, and *Botrytis tenula*. The power of fungi to destroy cellulose was also early suggested by Hartig in his studies on the destruction of woody tissues.

Later, the destruction of cellulose by fungi was observed in studies undertaken primarily to determine the causative agent of some common plant diseases. In this connection may be mentioned the work of DeBary with *Sclerotina libertiane*. Kissling in his biological studies of *Botrytis cinerea*, and Behrens with diseases of fruits.

The work of Van Iterson on the destruction of cellulose by fungi deserves special mention, as it gave the first indication of the extent of cellulose destruction by fungi. He was also the first to devise a method for isolating these organisms. To that purpose two sterile sheets of pure filter paper were placed in a Petri dish and moistened with the following solution:

Tap water	100.00 c. c.
Ammonium nitrate	.05 gram.
Potassium phosphate (monobasic)	.50 gram.

For inoculating material, soil or humus was used; however, the best results were obtained by exposing the dish to the air 12 hours and then cultivating at 24° C., taking care that the paper remained moist. After two or three weeks the paper was covered by a rich mold growth including a large number of species; among them were several species seldom found on malt gelatin, and on further study several of these were found to be active cellulose destroyers. The great abundance of these mold spores in the atmosphere was shown by the following experiment.

A Petri dish having a surface of 275 centimeters and containing filter paper moistened with the solution previously described was allowed to stand open in the garden 12 hours; 152 mold colonies developed, comprising 35 different species. It is evident from this experiment that large numbers of cellulose-destroying mold spores were floating in the air. The fungi found growing upon the paper were purified by means of malt gelatin. The destruction of cellulose

by pure cultures was then studied by inoculating sterile nutrient solutions containing pure filter paper prepared as previously described. In this work the following 15 species were isolated and described: *Sordaria humicola* Oud., *Pyronema confluens* Tul., *Chaetomium Kunzeanum* Zopf., *Pyrenochaeta humicola* Oud., *Chaetomella horrida* Oud., *Trichocladium asperum* Harz., *Stachybotrys alternans* Oud., *Sporotrichum bombycinum* (Corda) Rabh., *Sporotrichum roseolum* Oud. and Beijer., *Sporotrichum griseolum* Oud., *Botrytis vulgaris* Fr., *Mycogone puccinioïdes* (Preuss) Sacc., *Stemphylium macrosporoïdeum* (B. en Br.) Sacc., *Cladosporium herbarum* (Pers.) Link., and *Epiccocum purpurascens* Ehrenb.

Three years later the work of Appel appeared, which showed that certain forms of Fusarium can destroy cellulose with great rapidity. Ten grams of pure, dry filter paper were introduced into an Erlenmeyer flask, and 50 cubic centimeters of a nutrient solution containing potassium phosphate, magnesium sulphate, and potassium nitrate were added. An intensive cellulose fermentation developed, resulting in the destruction of 80 per cent of the paper in 14 days. Since all species of Fusarium can not use cellulose as a source of energy, the writer believes this fact can be made a valuable point in identifying the species of Fusarium.

In a series of experiments with a comparatively large number of molds Schellenberg showed that the destruction of the hemicellulose in plant tissues is sharply separated from the destruction of real cellulose, and also that molds act differently toward the hemicellulose of different plants. This difference in action is thought to be due to the differences in chemical composition of the plant tissues rather than to differences in solubility of the hemicelluloses. The results of this work are summarized in Table V.

TABLE V.—*Action of molds on real celluloses and hemicelluloses from various plants.*

Name of plant.	Cellulose from—		Hemicellulose from—				
	Cotton.	Hemp.	Molinia.	Lupinus.	Ruscus.	Phoenix.	Impatiens.
Mucor racemosus	−	−	+	−	−	−	−
Mucor neglectus	−	−	−	+	−	−	+
Mucor piriforme	−	−	−	+	−	−	+
Mucor globosus	−	−	+	+	−	−	+
Thamneidium elegans	−	−	−	+	−	−	+
Rhizopus nigricans	−	−	−	+	−	−	+
Penicillium glaucum (1)	−	−	−	−	−	−	+
Penicillium glaucum (2)	−	−	−	+	−	+	+
Sclerotinia fructigena	−	−	−	+	−	−	−
Sclerotinia cinerea	−	−	−	+	−	−	−
Botrytis cinerea	−	−	−	+	−	−	+
Botrytis vulgaris	−	−	+	+	−	−	+
Nectria cinnabarina	−	−	+	+	−	−	−
Cladosporium herbarum	−	−	+	+	−	−	+
Colletotrichum lindemuthianum	−	−	+	+	−	−	+
Trichothecium roseum	−	−	+	+	+	+	+

The recent work of Marshall Ward suggests the importance of Penicillium as a wood-destroying fungus. Spores of a pure culture of Penicillium were sown on sterile blocks of spruce wood; the mold grew freely and developed large quantities of spores on normal conidiophores. Sections of the wood showed that the hyphæ had entered the starch-bearing cells of the medullary rays of the sapwood and consumed the whole of the starch. In cultures three months old the hyphæ were found deep in the woody tissue passing from tracheid to tracheid via the border pits. In conclusion Ward says:

> It certainly looks as if Penicillium may be a much more active organism in initiating and carrying on the destruction of wood than has hitherto been supposed, and that it is not merely a hanger-on or follower of more powerful wood-destroying fungi.

Bourquelot has shown the great versatility of Aspergillus in the production of enzymes, having found it capable of producing invertase, maltase, trehalase, emulsin, inulase, diastase, and trypsin, and Bertrand and Holderer have found that it also produces cellase. Ward suggests that Penicillium may be equally rich in the capacity for enzyme production.

Among the higher fungi Schornstein found that *Poria vaillanti*, *Polyporus vaporarius*, *Polyporus destructor*, *Coniophora cerebella*, and *Paxillus panuoides* are capable of destroying wood, which, as is well known, is largely composed of cellulose. *Polyporus destructor* quickly forms fruiting bodies and never entirely destroys the wood. *Murulius lacrymans* and *M. pulverulentus* appeared on wood soon after it had been built into position and entirely destroyed it.

Arzberger in an investigation of the fungus which causes root tubercles on Ceanothus and Eleagnus found that it belonged to the genus Frankia and secreted an enzyme that destroyed the cell walls.

METHODS AND CULTURE MEDIA EMPLOYED.

In taking up the study of cellulose fermentation the elective cultural method employed by Omelianski and the method of Van Iterson of using sheets of filter paper were tried under both aerobic and anaerobic conditions. Microscopic examinations of the cultures kept under anaerobic conditions showed the presence of organisms similar to the hydrogen and methane ferments of Omelianski. In young cultures these organisms appeared only in small numbers, but became very numerous as the decomposition process advanced. In cultures kept until the paper had been completely destroyed, the spores of this organism became extremely numerous and microscopically the cultures appeared to be almost pure, but the presence of many other species was easily demonstrated by plating on ordinary media. The cultures kept under aerobic conditions showed no organisms

resembling the hydrogen or methane ferments which were so numerous in the anaerobic cultures, although the inoculum used was the same. The decomposition of the paper, however, was most rapid in the aerobic flasks. This was in accordance with Van Iterson's observations that cellulose may be rapidly decomposed by aerobic organisms. Plates from these cultures, like those grown anaerobically, showed the presence of several species of bacteria, even after numerous transfers. Our failure with these methods, together with Omelianski's admission of the impurity of his cultures after the most painstaking care to purify them, led us to believe that no accurate knowledge of cellulose fermentation could be obtained until a satisfactory plating medium had been secured.

In taking up the question of a suitable medium, a large number of nutrient solutions were first studied, including beef broth, decoctions of plant tissues, soil extracts, manure extracts, and numerous synthetic solutions. The following solution was finally adopted as giving the best results:

Potassium phosphate (dibasic)	1 gram.
Magnesium sulphate	1 gram.
Sodium carbonate	1 gram.
Ammonium sulphate	2 grams.
Calcium carbonate	2 grams.
Tap water	1,000 grams.

One hundred cubic centimeters of this solution is poured into a 200-cubic-centimeter Erlenmeyer flask and a single sheet of filter paper 10 centimeters in diameter folded so as to make a quarter circle is dropped into the solution. The paper should be in a loose fold, with the upper edge just beneath the surface of the solution. If the paper is not entirely immersed, the flask should be shaken until none of the paper is exposed. This precaution is necessary in order to keep down the mold growth which invariably occurs on the exposed paper. The flask is then plugged with cotton, sterilized, cooled, and inoculated with a very small quantity of the material under study and then gently shaken to insure an even distribution of the inoculum. The flasks are incubated at 30° C. until the fermentation process is well started, which generally takes from 5 to 10 days. The first signs of fermentation are usually indicated by the clouding of the solution, and a little later the paper takes on a dull, frayed appearance. A small quantity of the paper is now removed and placed in a control flask containing a small piece of sterile paper. If on agitation the paper from the inoculated flask is broken up more readily than that in the control the proper time for transfer has been reached. A small quantity of the paper is then removed by means of a small platinum spatula, placed in a second sterile flask, and the

flask agitated until the paper used as an inoculum is well broken up and distributed. Since the object of this transfer is to increase the number of cellulose ferments and at the same time crowd out the foreign species, it is important that the transfer be made as soon as the cellulose ferments have reached a high stage of development and before the by-products have become sufficiently abundant to allow a rapid development of foreign species. After three or four transfers a small piece of the paper is introduced into a flask containing sterile water and agitated until completely broken up. The necessary dilutions are then prepared and cellulose agar plates poured and kept at 30° C. in a moist chamber, which keeps the agar moist an indefinite time and is indispensable with slow-developing colonies. A convenient type of moist chamber may be prepared by inverting a large beaker over a shallow dish filled with water.

In many instances the cellulose ferments have cleared a well-defined zone in five or six days, but others may require three weeks or longer; however, the rapidity with which the cellulose agar is cleared is not always a safe index of the vigor of the organism, as some species which dissolve cellulose very slowly on solid media are very active in cellulose solutions.

It is seldom possible to secure a pure culture from the first set of plates, as even where the cellulose-fermenting colony is well separated from all other apparent growth it is often found to contain two or more species. To obtain a pure culture, all colonies are replated on cellulose agar before further study, and the replating is continued until only one type of colony appears on the plates and slides prepared from these colonies show the presence of only one kind of organism. Since the colonies on cellulose agar do not ordinarily form raised growths, it is often necessary to lift out a small piece of the agar containing the colony by means of a small platinum spatula. This spatula is also used to macerate the inoculum when it is introduced into the dilution flask. If a soft agar is used, the maceration can be accomplished readily by rubbing the agar against the side of the flask just above the surface of the water. An even distribution can then be effected by shaking.

Four varieties of special culture media have been found useful in studying the cellulose ferments. Their composition and method of preparation will now be described.

CELLULOSE AGAR.

Prepare one liter of a dilute ammonium-hydroxid solution by adding 3 parts water to 10 parts ammonium hydroxid, sp. gr. 0.90. Add a slight excess of copper carbonate and shake vigorously, allow to stand overnight, and then siphon off the supernatant solution.

Add 15 grams of unwashed sheet filter paper and shake occasionally until the paper is dissolved. Dilute to 10 liters and add slowly a 1 to 5 solution of hydrochloric acid, with vigorous shaking until the precipitation of the cellulose is complete. Dilute to 20 liters, allow the cellulose to settle, and decant the supernatant liquid. Wash by repeated changes of water, adding hydrochloric acid each time until the copper color disappers; then wash with water alone until the solution is free from chlorin. Allow it to settle several days and decant off as much of the clear solution as possible. If the percentage of cellulose is still too low, a portion of the solution is centrifugalized to bring the cellulose content up to 1 per cent.

Cellulose solution		500 c. c.
Agar		10 grams.
Nutrient solution, composed of—		
Potassium phosphate (dibasic)	1 gram.	500 c. c.
Magnesium sulphate	1 gram.	
Sodium chlorid	1 gram.	
Ammonium sulphate	2 grams.	
Calcium carbonate	2 grams.	
Tap water	1,000 c. c.	

STARCH AGAR.

To 800 cubic centimeters of boiling water add 10 grams of potato starch suspended in a little cold water. Concentrate by boiling to 500 cubic centimeters. This breaks up the starch grains and should give a nearly transparent starch solution.

Starch solution	500 c. c.
Agar	10 grams.
Nutrient solution (same as for cellulose agar)	500 c. c.

POTATO AGAR.

Pare, steam, and mash a quantity of potatoes. To 100 grams of mashed potato add 800 cubic centimeters of tap water and steam for one-half hour; filter through cotton.

Potato solution	500 c. c.
Agar	15 grams.
Nutrient solution (same as for cellulose agar)	500 c. c.

DEXTROSE AGAR.

Dextrose	10 grams.
Agar	15 grams.
Tap water	500 c. c.
Nutrient solution (same as for cellulose agar)	500 c. c.

THE OCCURRENCE AND GENERAL CHARACTERISTICS OF CELLULOSE-DESTROYING BACTERIA IN NATURE.

Preliminary examinations of sewer slime, of manures, and of the soils of the United States for cellulose-dissolving bacteria indicate that all decaying plant tissues are infested with these organisms and that numerous species occur, many of which have a wide distribution. Several species have been isolated again and again from widely separated regions. For instance, *Bacterium flavigena*, first isolated from Omelianski's hydrogen culture, has since been isolated from soils received from Hays, Kans., Arlington, Va., and Ponchatoula, La., while *Bacillus cytaseus* has been isolated from the soils of Jefferson, Me., Norfolk, Va., Rome, Ga., Alpena, Mich., and Riverside, Cal. All soil samples tested have been found to contain at least 1 species of cellulose-destroying bacteria, and as many as 3 species have been isolated from some. From the 12 soil samples examined 11 species have been isolated; in addition to these, 4 other species have been isolated from other sources, 1 of which belongs to the thermophile group. All soils examined have been found to contain thermophile bacteria capable of destroying cellulose very rapidly at 60° C. These organisms are especially abundant in manure heaps, and must play an important rôle in fermenting manures when high temperatures are reached.

In our investigations 15 species of cellulose-destroying bacteria have been isolated, all of which are morphologically and physiologically different from Omelianski's hydrogen and methane ferments. All of the organisms isolated grow upon ordinary gelatin, which in many cases is liquefied; however, gelatin is not a satisfactory medium for isolating these organisms, as it is in no way selective and some species soon lose their power to dissolve cellulose when grown upon it. Four species isolated from the soils of Utah grew more vigorously on gelatin than on any other solid medium, but after a few transfers on this medium the power to dissolve cellulose was entirely lost. This loss of cellulose-destroying power has also been noted with other species after several transfers on artificial media and is especially true of the thermophile organisms, which frequently lose their power to destroy cellulose after the first transfer on cellulose agar, although they continue to grow vigorously on ordinary media. All attempts to reestablish the normal activity of these organisms toward cellulose have been unsuccessful. No detailed descriptions of these species will be given, therefore, until their power to dissolve cellulose can be reestablished or they can be reisolated, and the descriptions written from cultures which act normally on cellulose agar.

After many trials to determine the rapidity of growth on the solid media used, we have found it advisable to give descriptions after 5,

10, and 15 days' incubation, the cellulose agar, potato slants, and gelatin stabs requiring the longer period. The quantity of acid produced by the different organisms in beef broth containing carbohydrates was determined according to the standard methods after incubation periods of 1, 2, and 3 days, but the quantities of acid produced during such short periods were too small to be of value; the titrations were therefore made after 6 and 12 days. The sugars, starch, and higher alcohols used were all broken down, with the production of more or less acid, with four exceptions: *Bacterium fimi* and *Bacterium liquatum* gave no acid in mannite, *Bacillus cytaseus* made no growth, and *Bacillus rossica*[1] gave an alkaline reaction. All except two of the organisms grew in litmus milk, most of which gave a faintly acid reaction in 2 days. Some of the species produced ammonia in Dunham's solution. In some cases nitrate was reduced to nitrite, but only one species carried the nitrite over to ammonia. Three of the organisms formed indol.

DESCRIPTIONS OF CELLULOSE-DESTROYING BACTERIA.

In describing the cultural characteristics of the cellulose-destroying bacteria our object has been to simplify the descriptions as much as possible by selecting media which would show the salient characteristics of the bacteria, and by using only those characters which remained fairly constant through three successive sets of cultures. In following this plan we have left out many data called for by the Society of American Bacteriologists, but the power to destroy cellulose is a test that places these organisms in a group by themselves, and it is believed the data given are sufficient for the identification of the organisms described. However, the descriptions are recognized as not entirely satisfactory, and as the study of the cultural characteristics of these and other cellulose-destroying organisms is continued it may be found advisable to add to the data now given.

BACTERIUM FIMI, N. SP.

(Pl. II, figs. 1, 2, and 3.)

I. *Morphology.*

1. Vegetative cells from 24-hour cultures at 30° C.
   Beef agar, average length 0.9 μ, maximum length 1.5 μ, width 0.4 μ. Dextrose agar, average length 1.4 μ, maximum length 2.8 μ, width 0.6 μ.
2. No endospores.
3. Staining reactions: Methylene blue +, carbol fuchsin +, Gram −.

II. *Cultural features.*

1. Agar strokes, 10 days. General characteristics: Glistening, smooth, moist growth, white to vitreous.
   Beef agar: Abundant, faint yellow.
   Potato agar: Abundant, iridescent.
   Dextrose agar: Moderate, iridescent.
   Starch agar: Abundant.
   Cellulose agar: Moderate.

[1] Described in Centralblatt für Bakteriologie [etc.], pt. 2, vol. 34, 1912, p. 492.

II. *Cultural features*—Continued.

2. Potato: After 15 days, moderate, glistening, smooth, cream colored; potato not colored.
3. Agar stab: Growth best at top, echinulate.
4. Gelatin stab: After 15 days liquefaction infundibuliform, becoming saccate in 50 days.
5. Beef broth: Slight clouding.
6. Litmus milk: Faintly acid in 2 days.
7. Plate cultures—

Cellulose agar, 15 days.

Form: Surface and bottom, round; embedded, lenticular or irregular.
Size: Surface and bottom, 1 to 4 mm.; embedded 0.7 to 1.8 mm. on major axis.
Enzymic zone: 0.3 to 1.6 mm.
Elevation: Raised.
Topography: Smooth.
Consistency: Slimy.
Chromogenesis: Surface and bottom, reflected light, gray with yellowish or whitish nucleus; transmitted light, brown. At angle of 45° by transmitted light bottom colonies show interior of colony bluish or iridescent and white ring around border. Embedded, reflected light, white; transmitted light, opaque.
Internal structure: Surface and bottom granular with opaque to translucent granular nucleus, and frequently having finely granular ring at border; embedded, opaque, often with numerous outgrowths.
Edge: Entire to undulate.

Potato agar, 5 days.

Form: Surface and bottom, round; embedded, lenticular to triangular.
Size: Surface and bottom, 1 to 6 mm.; embedded, 0.5 to 1.5 mm.
Elevation: Convex.
Topography: Smooth.
Consistency: Slimy.
Chromogenesis: Surface, reflected light, glistening grayish white; transmitted light, opaque or translucent brownish gray, often with opaque nucleus. Embedded, reflected light, white; transmitted light, opaque. Bottom, reflected light, gray; transmitted light, light brown, at 45° bluish and often iridescent.
Internal structure: Surface, homogenous, opaque or finely granular, often with lenticular or round nucleus; embedded, opaque, sometimes with translucent irregular, finely granular outgrowths; bottom, homogenous, finely granular, frequently with granular nucleus.
Edge: Entire.

Beef agar, 5 days.

Form: Surface and bottom, round; embedded, lenticular.
Size: Surface and bottom, 1 to 4 mm.; embedded, 0.6 to 1.5 mm. on major axis.
Elevation: Convex.
Topography: Smooth.
Consistency: Slimy.
Chromogenesis: surface and bottom, reflected light, yellowish or grayish white; transmitted light, translucent brown; at 45° iridescent ring at border. Embedded, reflected light, yellowish or grayish white; transmitted light, opaque.
Odor: None.

II. *Cultural features*—Continued.

7. Plate cultures—Continued.

Beef agar, 5 days—Continued.

Internal structure: Surface, granular, often with lenticular nucleus and finely granular and hyaline at edge; embedded, opaque; bottom, granular, sometimes with nucleus.

Edge: Entire.

Starch agar, 5 days.

Form: No surface colonies; embedded, lenticular to irregularly round; bottom, round.

Size: Embedded, 0.4 to 1.2 mm.; bottom, 1 to 1.5 mm.

Enzymic zone: 1.7 to 3 mm.

Elevation: No surface growth, but agar raised by colonies just below surface.

Chromogenesis: Embedded, reflected light, white; transmitted light, opaque. Bottom, reflected light, opalescent or white; transmitted light, barely translucent, dark gray.

Internal structure: Embedded, opaque and often irregular with outgrowths; bottom, granular, generally becoming finely granular at edge, and usually with lenticular nucleus.

Edge: Entire.

Dextrose agar, 5 days.

Form: Surface and bottom, round; embedded, lenticular.

Size: Surface and bottom, 0.8 to 1.2 mm.; embedded, 0.4 to 1 mm.

Elevation: Convex.

Topography: Smooth.

Consistency: Slimy.

Chromogenesis: Surface, reflected light, grayish white, generally with white nucleus; transmitted light, translucent brownish gray. Embedded, reflected light, white; transmitted light, opaque. Bottom, reflected light, gray; transmitted light, smoky brown.

Internal structure: Surface and bottom, finely granular, usually with round or lenticular nucleus; embedded, opaque, granular at edge.

Edge: Entire.

III. *Physical and biochemical features.*

1. Peptone water—

Test for—	Addition to peptone water.						
	Dextrose.	Lactose.	Saccharose.	Maltose.	Glycerin.	Mannite.	Starch.
Gas production	0	0	0	0	0	0	0
Acid production, 6 days	1.42	.70	1.60	1.45	.53	0	1.48
Acid production, 12 days	1.60	.90	1.63	1.43	.76	0	1.62

2. Dunham's: Ammonia trace.

3. Nitrate broth: Nitrite +; ammonia −.

4. Peptone-nitrite solution: Indol +.

BACTERIUM LIQUATUM, N. SP.

(Pl. I, figs. 1, 2, and 3.)

I. *Morphology.*

1. Vegetative cells from 24-hour cultures at 30° C. Beef agar, average length 1.7 μ, maximum length 2.6 μ, width 0.4 μ. Potato agar, average length 0.8 μ, maximum length 1.5 μ, width 0.3 μ.

2. No endospores.

3. Staining reactions: Methylene blue +; carbol fuchsin +; Gram −.

II. *Cultural features.*

1. Agar strokes, 10 days. General characteristics: Glistening, smooth, moist growths.
   Beef agar: Abundant, raised, grayish yellow; agar whitened.
   Potato agar: Abundant, flat, watery, pale grayish yellow.
   Dextrose agar: Scant, flat, watery, vitreous to pale yellow.
   Starch agar: Moderate, flat, vitreous to pale yellow.
   Cellulose agar: Moderate, convex, vitreous to pale yellow.
2. Potato: After 15 days growth abundant, glistening, smooth, brilliant canary yellow; potato not colored.
3. Agar stab: Growth best at top, papillate.
4. Gelatin stab: After 15 days liquefaction napiform; after 50 days stratiform with liquefied gelatin present.
5. Beef broth: Moderate clouding, scant compact sediment.
6. Litmus milk: Faintly acid in two days.
7. Plate cultures—
   Cellulose agar, 15 days.
      Form: Surface and bottom, round; embedded, lenticular to irregularly round.
      Size: Surface, 1 mm.; embedded, 1 mm. on major axis; bottom, 1.5 mm.
      Enzymic zone: 0.4 to 0.75 mm. wide and slightly depressed.
      Elevation: Raised or umbilicate. Embedded colonies just below surface give umbonate appearance due to depression of enzymic zone.
      Topography: Smooth.
      Consistency: Soft.
      Chromogenesis: Surface, embedded and bottom, reflected light, faint yellowish gray; surface and bottom, transmitted light, translucent gray generally with opaque nucleus, surface sometimes showing opaque ring at edge; embedded, opaque.
      Internal structure: Surface and bottom, granular, generally with round or lenticular nucleus; embedded, opaque, sometimes conglomerate.
      Edge: Surface and bottom, irregular, finely granular; embedded, entire.
   Starch agar, 5 days.
      Form: Round.
      Size: Embedded and bottom, 0.5 to 2 mm.
      Enzymic zone: 1 mm.
      Elevation: No surface colonies. Medium may be raised by embedded colonies just below surface.
      Chromogenesis: Embedded, reflected light, white, opaque. Bottom, reflected light, light gray with whitish gray nucleus; transmitted light, gray with opaque nucleus or like embedded.
      Internal structure: Embedded and bottom, central area opaque, becoming coarsely granular near edge.
      Edge: Embedded, irregular, granular; bottom, blending with medium or irregularly granular.
   Beef agar, 5 days.
      Form: Surface and bottom, round; embedded, lenticular to round.
      Size: Surface, 1.5 to 3 mm.; embedded, 0.5 to 1 mm.; bottom, 1 to 1.5 mm.
      Elevation: Convex to pulvinate.
      Topography: Smooth.
      Consistency: Slightly viscid.

II. *Cultural features*—Continued.

7. Plate cultures—Continued.

Beef agar, 5 days—Continued.

Chromogenesis: Surface, reflected light, glistening, sebaceous; transmitted light, translucent brown, some with vitreous edge. Embedded, reflected light, sebaceous; transmitted light, opaque. Bottom, reflected light, gray; transmitted light, translucent brown.

Odor: None.

Internal structure: Surface, coarsely granular, generally showing irregular granular nucleus; embedded, opaque, often showing outgrowths in all directions; bottom, granular, with a granular nucleus.

Edge: Entire.

Potato agar, 5 days.

Form: Surface and bottom, round; embedded, lenticular.

Size: Surface, 1 to 5 mm.; embedded, 0.5 to 1.5 mm. on major axis; bottom, 1 mm., sometimes spreading to 15 mm.

Elevation: Convex.

Topography: Smooth.

Consistency: Watery.

Chromogenesis: Surface, reflected light, glistening, opalescent; transmitted light, edge bluish and iridescent. Embedded, reflected light, cream color; transmitted light, opaque. Bottom, reflected light, gray; transmitted light, vitreous, sometimes with a brownish central area.

Internal structure: Surface, granular, often almost hyaline, generally showing nucleus; embedded, granular, sometimes showing numerous outgrowths; bottom, finely granular, generally with granular nucleus and often having grumose center.

Edge: Entire.

Dextrose agar, 5 days.

Form: Surface and bottom, round; embedded, lenticular.

Size: Surface and bottom, 1 to 2 mm.; embedded, 0.5 to 0.75 mm.

Elevation: Convex.

Topography: Smooth.

Consistency: Watery.

Chromogenesis: Surface, reflected light, vitreous to gray, with whitish gray central area often showing white nucleus; transmitted light, translucent light brownish gray, with vitreous edge and opaque nucleus. Embedded, reflected light, grayish white; transmitted light, opaque; bottom, like surface, no nucleus.

Internal structure: Surface, finely granular, usually with opaque lenticular nucleus; embedded, opaque, but showing granular at edge; bottom, finely granular with small granular nucleus.

Edge: Entire.

III. *Physical and biochemical features.*

1. Peptone water—

Test for—	Addition to peptone water.						
	Dextrose.	Lactose.	Saccharose.	Maltose.	Glycerin.	Mannite.	Starch.
Gas production	0	0	0	0	0	0	0
Acid production, 6 days	1.20	.50	1.32	1.16	.08	0	1.38
Acid production, 12 days	1.27	.98	1.33	1.16	.23	0	1.40

II. *Physical and biochemical features*—Continued.
2. Dunham's: Ammonia +.
3. Nitrate broth: Nitrites +; ammonia −.
4. Peptone-nitrite solution: Indol +.

BACILLUS BIBULUS, N. SP.

(Pl. II, figs. 4, 5, and 6, and Pl. IV, figs. 1 and 2.)

I. *Morphology.*
1. Vegtative cells from 24-hour cultures at 30° C. Beef agar, average length 1.3 μ, maximum length 2.0 μ, width 0.4 μ. Dextrose agar, average length 0.8 μ, maximum length 1.4 μ, width 0.4 μ.
2. No endospores.
3. Staining reactions: Methylene blue +; carbol fuchsin +; Gram −.

II. *Cultural features.*
1. Agar strokes, 10 days. General characteristics: Glistening, smooth, moist, raised or convex growth.
   Beef agar: Abundant, grayish yellow.
   Potato agar: Abundant, grayish yellow.
   Dextrose agar: Scant, white.
   Starch agar: Scant, vitreous to gray.
   Cellulose agar: Moderate, yellowish.
2. Potato: After 15 days' growth abundant, glistening, smooth, brilliant canary yellow. Potato not colored.
3. Agar stab: Growth best at top, papillate.
4. Gelatin stab: After 15 days' line of puncture filiform, later echinulate; liquefaction crateriform. After 50 days deeply crateriform, no liquefied gelatin present.
5. Beef broth: Slight clouding, scant compact sediment.
6. Litmus milk: Faintly acid in two days.
7. Plate cultures—
   Cellulose agar, 15 days.
      Form: Surface and bottom, round; embedded, round to irregularly round.
      Size: Surface and bottom, 0.5 to 0.8 mm.; embedded, 0.3 to 0.5 mm.
      Enzymic zone: 0.3 mm. in some cases.
      Elevation: Convex.
      Topography: Smooth.
      Consistency: Soft.
      Chromogenesis: Surface and bottom, reflected and transmitted light, opalescent, usually with grayish white opaque nucleus. Embedded, reflected light, grayish or yellowish white, transmitted light, opaque.
      Internal structure: Surface granular, sometimes with clouded radial areas extending to edge of colony or with a grumose center; embedded, granular, may show lenticular mother growth with numerous outgrowths; bottom, granular, often with roundish granular nucleus, and may also be clouded.
      Edge: Irregular and granular.
   Starch agar, 5 days.
      Form: No surface growth; embedded, irregularly round to round; bottom, round.
      Size: Embedded and bottom, 0.3 to 2.5 mm.
      Enzymic zone: 1 to 2.5 mm.
      Elevation: Medium slightly raised by colonies just under the surface.
      Chromogenesis: Embedded and bottom, reflected light, white; transmitted light, translucent gray to opaque.

II. *Cultural features*—Continued.
7. Plate cultures—Continued.
Starch agar, 5 days—Continued.
Internal structure: Embedded, narrow finely granular zone around edge, remainder opaque; bottom, finely granular zone at edge wider than embedded; remainder opaque.
Edge: Embedded, entire to irregularly granular; bottom, blending with medium.
Dextrose agar, 5 days.
Form: Surface and bottom, round; embedded, lenticular.
Size: Surface and embedded, 0.4 to 0.6 mm.; bottom, 0.5 to 1 mm.
Elevation: Convex.
Topography: Smooth.
Consistency: Slimy.
Chromogenesis: Surface, reflected light, white to faint yellowish gray; transmitted light, barely translucent, dark brown. Embedded, reflected light, like surface; transmitted light, opaque; bottom, reflected light, opalescent.
Internal structure: Surface, finely granular, with an opaque round or granular nucleus; embedded, opaque; bottom, finely granular, sometimes with nucleus.
Edge: Entire.
Beef agar, 5 days.
Form: Surface and bottom, round; embedded, lenticular.
Size: Surface and bottom, 1 to 2 mm.; embedded, 0.5 to 1 mm. on major axis.
Elevation: Convex.
Topography: Smooth.
Consistency: Soft.
Chromogenesis: Surface, reflected light, light yellow; transmitted light, translucent brownish yellow, may have opaque nucleus. Embedded reflected light, yellow to yellowish gray; transmitted light, opaque. Bottom, reflected light, gray; transmitted light, translucent smoky brown.
Internal structure: Surface, granular, with dark round or lenticular nucleus, sometimes with hyalin edge; embedded, granular, occasionally with outgrowths in one or two planes; bottom, granular, usually with small granular nucleus, and often with edgelike surface.
Odor: None.
Edge: Entire.
Potato agar, 5 days.
Form: Surface and bottom, round; embedded, lenticular.
Size: Surface and bottom, 0.5 to 1.5 mm.; embedded, 0.5 to 0.8 mm. on major axis.
Elevation: Pulvinate.
Topography: Smooth.
Consistency: Soft.
Chromogenesis: Surface, reflected light, yellowish gray; transmitted light, barely translucent brown. Embedded, reflected light, yellow; translucent light, opaque. Bottom, reflected light, gray; transmitted light, translucent brown.
Internal structure: Surface, finely granular, generally with round or lenticular opaque or granular nucleus, often with hyalin edge; embedded, opaque; bottom, like surface except nucleus, which if present is small, round, and granular.
Edge: Entire.

III. *Physical and biochemical features.*

1. Peptone water—

Test for—	Addition to peptone water.						
	Dextrose.	Lactose.	Saccharose.	Maltose.	Glycerin.	Mannite.	Starch.
Gas production	0	0	0	0	0	0	0
Acid production, 6 days	1.75	1.20	1.57	1.22	.15	.75	1.90
Acid production, 12 days	1.85	1.28	1.50	1.47	.35	1.20	2.07

2. Dunham's: Ammonia +.
3. Nitrate broth: Nitrites −; ammonia −.
4. Peptone-nitrite solution: Indol, trace.

PSEUDOMONAS SUBCRETUS, N. SP.

(Pl. I, figs. 4, 5, and 6.)

I. *Morphology.*

1. Vegetative cells: Starch agar, 24 hours at 30° C., average length 1.2 μ. Maximum length 1.4 μ, width 0.3 μ. Beef agar, 48 hours at 30° C. (no growth in 24 hours), average length 1.4 μ, maximum length 3.0 μ, width 0.4 μ.
2. No endospores.
3. Staining reactions: Methylene blue +; carbol fuchsin +; gram −.

II. *Cultural features.*

1. Agar strokes, 10 days. General characteristics; glistening, smooth, moist, vitreous to faint yellow.
   Beef agar: Moderate, flat.
   Potato agar: No growth.
   Dextrose sugar: Scant.
   Starch agar: Moderate.
   Cellulose agar: No surface growth. Moderate, generally faint yellow growth in medium, area of growth sunken.
2. Potato: After 15 days' growth scanty, concave due to slight liquefaction of potato, white to faint yellow. Potato bleached around growth.
3. Agar stab: Growth best at top, papillate.
4. Gelatin stab: After 15 days filiform, no liquefaction.
5. Beef broth: No growth.
6. Litmus milk: No growth.
7. Plate cultures—
   Cellulose agar, 15 days.
      Form: Round.
      Size: Average 3 mm.
      Enzymic zone: Enzyme acts within the colony; older colonies may show narrow clear zone about 0.3 mm. wide.
      Elevation: Medium concave, no surface growth.
      Chromogenesis: Reflected light, transparent gray or yellowish gray; transmitted light, smoky brown.
      Internal structure: Central area clouded light brown, then a zone like the medium but less dense, sometimes surrounded by a denser border line which may be broken.
      Edge: Entire to undulate.
   Starch agar, 5 days.
      Form: Round.
      Size: Surface and bottom, 0.3 to 1.5 mm.; embedded, 0.5 to 0.7 mm.
      Enzymic zone: 2 to 4 mm.
      Elevation: Convex.

II. *Cultural features*—Continued.

7. Plate cultures—Continued.

Starch agar, 5 days—Continued.

Topography: Smooth.

Consistency: Very soft.

Chromogenesis: Surface, reflected light, yellowish white; transmitted light, opaque nucleus surrounded by narrow or wide translucent yellow zone. Embedded, reflected light, yellowish white; transmitted light, opaque. Bottom, reflected light, gray with grayish white nucleus; transmitted light, translucent yellowish gray with opaque nucleus.

Internal structure: Surface, granular brownish nucleus surrounded by finely granular light-brown zone often almost hyalin at edge; embedded, opaque with narrow, finely granular edge; bottom, opaque or nearly opaque, brown, granular central area surrounded by light-brown granular zone, often with narrow lighter ring at edge, the latter often finely granular.

Edge: Surface and bottom, entire; embedded, irregularly granular or shading off into medium.

Dextrose agar, 5 days.

Form: Surface and bottom, round; embedded, lenticular.

Size: Surface and bottom, 1 to 1.3 mm.; embedded, 0.4 mm.

Elevation: Convex.

Topography: Smooth.

Consistency: Soft.

Chromogenesis: Surface, reflected light, yellowish white; transmitted light, translucent light brown. Embedded, reflected light, yellowish white; transmitted, opaque. Bottom, reflected light, gray; transmitted light, translucent light brown.

Internal structure: Surface, finely granular, generally with small granular nucleus. Embedded, coarsely granular; bottom, central granular area usually becoming finely granular around the edge and having small granular nucleus.

Edge: Entire.

Beef agar, 5 days.

Form: Irregularly round.

Size: Surface and bottom, 0.7 to 1.4 mm.; embedded, 0.4 to 0.7 mm.

Elevation: Convex, often arising from depression in agar.

Topography: Smooth.

Consistency: Soft.

Odor: None.

Chromogenesis: Surface, reflected light, faint yellowish gray; transmitted light, translucent light brown. Embedded, reflected light, faint yellowish gray; transmitted light, opaque. Bottom, transmitted light, light brown generally with small opaque nucleus and ring near edge.

Internal structure: Surface, finely granular, with round or oval dark-brown barely translucent nucleus; sometimes growth so dense nucleus can not be distinguished. Embedded, large central area opaque or occasionally translucent with narrow, translucent, finely granular edge. Bottom, granular, usually almost opaque at center.

Edge: Surface and bottom, entire or granular and blending with medium; embedded, entire.

Potato agar, 5 days.

No growth.

III. *Physical and biochemical features.*

1. Peptone water—

Test for—	Addition to peptone water.						
	Dextrose.	Lactose.	Saccharose.	Maltose.	Glycerin.	Mannite.	Starch.
Gas production	0	0	0	0	0	0	0
Acid production, 6 days	.35	.22	.05	.52	0	0	.60
Acid production, 12 days	.57	.53	.05	.52	0	0	.60

2. Dunham's: Ammonia −.
3. Nitrate broth: Nitrite trace; ammonia −.
4. Peptone-nitrite solution: Indol −.

BACILLUS CYTASEUS, N. SP.

(Pl. III, figs. 1, 2, 3, 4, and 5, and Pl. IV, figs. 3 and 4.)

I. *Morphology.*

1. Vegetative cells from 24-hour cultures at 30° C. Cellulose agar, average length 1.8 μ, maximum length 3.4 μ, width 0.2 μ. Potato agar, average length 2.7 μ, maximum length, 5.0 μ, width 0.5 μ.
2. Endospores: Form elliptical; limits of size, 1.4 μ by 0.7 to 2.6 μ by 1 μ; size of majority, 2μ by 0.9 μ. Spores withstand 80° C. for 15 minutes.
3. Involution forms on potato agar in 2 days at 30° C.
4. Staining reactions: Methylene blue +; carbol fuchsin +; gram −.

II. *Cultural features.*

1. Agar strokes, 10 days. General characteristics: Flat, vitreous to faint gray growth.
   Beef agar. No growth.
   Potato agar: Abundant, glistening.
   Dextrose agar: Abundant, dry, with oily luster.
   Starch agar: Abundant, glistening.
   Cellulose agar: No raised growth. Moderate growth under surface with shallow, clear stratum below.
2. Potato: After 15 days no growth.
3. Agar stab: Echinulate.
4. Gelatin stab: After 15 days very scant growth on surface with few isolated beads just below the surface.
5. Beef broth: No growth.
6. Litmus milk: No growth.
7. Plate cultures—
   Cellulose agar, 15 days.
   Form: Surface and bottom, irregularly round; embedded, round.
   Size: Surface, 1.5 to 5 mm.; embedded, 0.5 to 0.8 mm.; bottom, 12 to 25 mm.
   Enzymic zone: 0.5 to 1 mm.
   Elevation: Flat.
   Topography: Smooth.
   Consistency: Soft.
   Chromogenesis: Surface, reflected light, light gray; transmitted light, transparent gray. Embedded, reflected light, gray, often with narrow white border and very small nucleuslike border; transmitted light, translucent brownish gray with small, almost opaque nucleus. Bottom, reflected and transmitted light, vitreous.

II. *Cultural features*—Continued.

7. Plate cultures—Continued.

Cellulose agar, 15 days—Continued.

Internal structure: Surface, homogeneous, granular, with small raised nucleus; embedded, granular, often may be coarsely granular around edge; bottom granular, generally no nucleus, and often with faint, irregular, clouded areas.

Edge: Surface, lobate; embedded, regular and granular; bottom, regular and granular, or blending with medium.

Potato agar, 5 days.

Form: Surface and bottom, irregular; embedded, lenticular.

Size: Surface and bottom, 6 to 30 mm.; embedded, 0.2 to 0.4 mm. on major axis.

Elevation: Flat.

Topography: Beaded.

Consistency: Soft.

Chromogenesis: Surface and bottom, vitreous, often with whitish gray edge; embedded, reflected light, white; transmitted light, opaque.

Internal structure: Surface, a spreading colony of granular beads becoming denser and fusing at edge; embedded, granular; bottom, spreading, granular.

Edge: Surface, irregular, beaded; embedded, entire; bottom, irregular often ameboid.

Starch agar, 5 days.

Form: No surface growth; embedded, round; bottom, round or irregularly round.

Size: Embedded, 1 to 7 mm.; bottom, 3 to 10 mm.

Enzymic zone: 2 to 5 mm.

Chromogenesis: Embedded, reflected light, whitish gray, generally with concentric whitish rings near border; transmitted light, translucent, faint, brownish gray. Bottom, reflected light, faint gray or vitreous; transmitted light, faint brown or vitreous.

Internal structure: Embedded, granular, generally more coarsely granular at edge, often with rings near border; bottom, finely granular, with growth generally denser around edge and often with small granular nucleus.

Edge: Embedded, irregularly granular; bottom, granular, sometimes auriculate to fimbriate.

Dextrose agar, 5 days.

Punctiform colonies, growth not typical.

Beef agar, 5 days.

Colonies very small, growth not typical.

III. *Physical and biochemical features.*

1. Peptone water—

Test for—	Addition to peptone water.						
	Dextrose.	Lactose.	Saccharose.	Maltose.	Glycerin.	Mannite.	Starch.
Gas production	0	0	0	0	0	0	0
Acid production, 6 days	0	0	0	0	0	0	0
Acid production, 12 days	0	0	0	0	0	0	0

2. Dunham's: Ammonia −.

3. Nitrate broth: Nitrites −; ammonia −.

4. Peptone-nitrite solution: Indol −.

CELLULOSE DESTRUCTION BY FILAMENTOUS FUNGI.

From early studies of cellulose fermentation in our laboratory of soil bacteriology it became apparent that many species of filamentous fungi are capable of destroying cellulose very rapidly. This was first indicated by a series of experiments designed primarily to test the cellulose-dissolving power of various soils and also to study the value of cellulose as a source of energy for nitrogen-fixing organisms. Pure filter paper was cut into small squares, approximately 2 millimeters, and evenly distributed through the soil; 200-gram samples of soil were used, to which 2 per cent of paper was added; as much water as the soil would hold without becoming sticky was then added, and after a thorough mixing the samples were held at 30° C. The small pieces of paper soon became covered with mold growths, including many species. In plating out from these soils on cellulose agar after 30 days' incubation, in one instance as many as 200,000,000 mold colonies were obtained from a gram of dry soil. The check sample at the beginning of the experiment gave less than 20,000 mold colonies, and after 30 days' incubation the number had increased to 100,000. Thus, it appears that the sample to which cellulose had been added contained 2,000 times as many mold spores capable of growing on cellulose agar as the check sample. It is therefore obvious that cellulose either directly or indirectly stimulated the development of molds in the soil.

This stimulation might be explained on the theory that the molds were able to utilize the by-products of cellulose decomposition caused by the activity of bacteria; but on examining the mold colonies on the cellulose-agar plates it was found that about 40 per cent of them had cleared up a well-defined zone around the colony, thus showing their cellulose-dissolving power, while a careful search failed to reveal a single cellulose-dissolving bacterial colony. It therefore appears that the great development in the mold flora was due, in part at least, to the presence of numerous species of cellulose-dissolving molds; but as an increase in the number of noncellulose-dissolving molds was also found, the conclusion was reached that a great many species of molds which were able to use the by-products produced by the fermentation of the cellulose were also present. The failure to obtain cellulose-destroying bacteria from this soil by direct plating does not mean that these organisms were not present in considerable numbers in this soil. Owing to the large number of mold colonies developing on the plates it was necessary to prepare plates from very high dilutions, and as the mold colonies ordinarily develop more readily than the bacterial colonies the detection of the latter was uncertain, even when present in considerable numbers. In fact, the presence of cellulose-dissolving bacteria was easily demonstrated by

dropping a small quantity of the soil into a nutrient solution containing filter paper and handling after the manner previously described for isolating cellulose-destroying bacteria.

A second experiment was planned to determine the presence of the cellulose-dissolving mold spores in the atmosphere. Ordinary Petri dishes containing sterile cellulose agar were exposed to the laboratory air for five minutes and then incubated at 30° C. for six days. From 8 to 25 mold colonies developed, a high percentage of which cleared a well-defined zone about the colony, and some destroyed the cellulose with such rapidity that the entire plate of cellulose agar was cleared up at the end of the sixth day.

The power of *Penicillium africanum, P. pinophilum,* an unnamed new species of Penicillium, and *Aspergillus fumigatus* to dissolve different forms of cellulose, was also tested. These species had previously shown a rapid destruction of the precipitated cellulose in the regular cellulose agar. Cellulose in the form of cotton, precipitated filter paper, rye straw, cherry-wood shavings, and cedar-wood shavings was placed in large Petri dishes and moistened with the regular cellulose nutrient solution. The experiment was continued three months at room temperature, care being taken to keep all the material under study well moistened. At the end of the experiment the differences shown in Table VI were observed.

TABLE VI.—*Action of Penicillium and Aspergillus on different forms of cellulose.*

Material tested.	Penicillium africanum.	Penicillium pinophilum.	Penicillium not named.	Aspergillus fumigatus.
Precipitated cellulose.	Good growth; cellulose dissolved.	Good growth; cellulose dissolved.	Good growth; cellulose dissolved.	Good growth; cellulose dissolved.
Cotton	do	do	do	Do.
Rye straw	Good growth; cellulose not attacked.	Poor growth; cellulose not attacked.	Good growth; cellulose not attacked.	Good growth; cellulose not attacked.
Cherry shavings	Poor growth; cellulose not attacked.	No growth	Poor growth; cellulose not attacked.	Poor growth; cellulose not attacked.
Cedar shavings	do	Poor growth; cellulose not attacked.	No growth	Do.

The cotton was reduced to a transparent gelatinous mass and was stained a reddish brown color by all molds except by *Penicillium* n. sp. (not named). Although the molds made a good growth on rye straw they did not appear to attack the walls of the cells nor did they show any action on the wood shavings from cherry or cedar.

With the exception of the Penicillium and Aspergillus forms, only a few of the species isolated have been identified. For the identification of the species named below we are indebted to Dr. Charles Thom, Mycologist in Charge of Cheese Investigations, Bureau of Animal Industry, United States Department of Agriculture.

Penicillium expansum (Link) Thom.
Penicillium pinophilum Hedg.
Aspergillus nidulans Eidam.
Aspergillus fumigatus Fres.
Aspergillus flavus Link.
Gliocladium viride Matr.
Trichoderma lignorum (Tode) Harz.
Penicillium africanum Doebelt.
Penicillium n. sp.
Penicillium n. sp.

Dr. Thom has also kindly furnished us a set of his Penicillium cultures described in his bulletin.[1] These have been tested for their cellulose-dissolving power, and the following species have been found to destroy cellulose more or less rapidly:

Penicillium claviforme Bainier. Destruction strong.
pinophilum Hedg. Destruction weak.
luteum Zukal. Destruction strong.
Penicillium roseum Link. Destruction medium.
stoloniferum Thom. Destruction strong.
rugulosum Thom. Destruction strong.

Mrs. Flora W. Patterson, Mycologist of the Bureau of Plant Industry, has identified the following species:

Cephalothecium roseum Cda.
Haplographium echinatum (Riv.) Sacc.
Sporotrichum radicicolum A. Zimm.
Sporotrichum thebaicum Ehrenb.
Sporotrichum sporulosum Sacc.
Fusarium sp. undet.

Contrary to the general belief that soil fungi are largely confined to acid soils, the writers have found that the cellulose-dissolving forms multiply with great rapidity in alkaline soils when cellulose in the form of filter paper was added.

While only preliminary studies of cellulose destruction in our soils have been made, the great variety and vigor of the cellulose-dissolving fungi lead to the conclusion that cellulose destruction in soil is due in a large measure to the activity of these organisms, and it is certain that their importance in this connection has been greatly underestimated.

PRODUCTS RESULTING FROM THE DESTRUCTION OF CELLULOSE BY BACTERIA.

In view of the fact that the cellulose added to the soil represents a large amount of potential energy, the value of which depends upon the nature of the compounds formed in its destruction, it becomes quite interesting to inquire into the nature of these substances. If we are to accept the conclusions of earlier investigators, a large part of the energy contained in the cellulose is invariably lost in the form of gaseous products; however, the failure of any of the cellulose-dissolving bacteria studied in our laboratory to produce gaseous products from cellulose or sugar solutions in which they made a

[1] Thom, Charles. Cultural Studies of Species of Penicillium. Bulletin 118, Bureau of Animal Industry, U. S. Dept. of Agriculture. 1910.

luxuriant growth would seem to indicate that no gas is formed by pure cultures of the cellulose ferments.

Under normal conditions the compounds formed by the cellulose ferments will of course be seized upon by a host of other microorganisms and split up into simpler compounds. In some sandy soils the destruction may be extremely rapid and complete, resulting in the formation of little or no humus; under such conditions the carbon content of the cellulose is no doubt liberated as carbon dioxid, and under other conditions a large percentage of the carbon may be lost as methane. In either case the loss is believed to be due to secondary fermentations induced by organisms acting upon the by-products of the cellulose ferments.

It is well known that many fermentation processes may result in the formation of carbon dioxid and hydrogen and that such fermentations may be induced by many species of bacteria. In our investigations of the by-products of cellulose fermentation many experiments have been conducted to determine the nature of the gaseous products formed by mixed cultures of cellulose ferments. In a large series of flasks the sterile nutrient solution containing a quantity of pure filter paper was inoculated with pure cellulose ferments; a disintegration of the paper always occurred without any gas formation. The flasks were then divided into two series. The first series was left uncontaminated, while each flask of the second series was inoculated with a very small quantity of soil, manure, or sewage slime. In 48 hours after contamination all of the flasks of the second series had produced small gas bubbles; the gas formation continued from two to four weeks, during which time 100 cubic centimeters or more of gas was collected. None of the flasks of the uncontaminated series showed any gas, although the disintegration of the paper was almost complete. An analysis of the gas formed showed it to consist of a mixture of carbon dioxid and hydrogen.

In no case has a methane fermentation been secured from pure filter paper in the cellulose nutrient solution, but only from fermenting solutions rich in nitrogenous organic matter, and then only when the solution has been held under strict anaerobic conditions. A quantity of fresh horse manure was placed in a 2-liter filter flask, the flask filled with tap water, and the air exhausted to produce strictly anaerobic conditions. The flask was then incubated at 37° C. for 16 days before any gas formation was evident, but when once started the formation was very rapid and about 40 cubic centimeters of gas was produced a day. An analysis of the gas produced during the first seven days showed a large percentage of carbon dioxid and hydrogen with a little nitrogen; a later analysis, however, gave a large percentage of carbon dioxid, methane, and only a

little hydrogen and nitrogen. The experiment was repeated at 55° C. The gas formation started in 24 hours and continued vigorously for 6 days, after which it ceased, although a considerable quantity of cellulose remained undissolved. An analysis of the gas showed it to consist of carbon dioxid, hydrogen, and nitrogen.

Considerable time has been spent in an effort to isolate the organisms giving rise to the gaseous products in the cellulose solutions, and while several organisms have been isolated which form gas in sugar solutions none has been discovered which will form gas from cellulose when grown in association with the cellulose ferments. Future investigations, however, will no doubt reveal many organisms, which have this power. Such investigations can be more intelligently carried on when we know the nature of the compounds formed by the cellulose ferments. While it has been possible to make only preliminary examinations of these compounds from rather small quantities of solutions, sufficient data have been secured to warrant the conclusion that the principal by-products of some species of cellulose-dissolving bacteria consist of formic and acetic acid, while with other species only traces of fatty acids are formed. None of the solutions examined have shown any trace of aldehydes, ketones, alcohols, or carbohydrates capable of reducing Fehling's solution. Since the by-products of cellulose decomposition apparently vary with the species of bacteria causing such decomposition, an accurate knowledge of the compounds formed will no doubt be of much value in specific classification of these organisms.

CONCLUSIONS.

(1) Neither the elective culture method of Omelianski nor the filter-paper sheet method of Van Iterson is satisfactory for isolating the cellulose-dissolving bacteria, but these organisms may be isolated readily by means of a selective medium such as cellulose agar.

(2) Cellulose-destroying bacteria and cellulose-destroying molds are universally present in cultivated soils.

(3) The cellulose-destroying bacteria are facultative in nature but destroy cellulose most rapidly in the presence of air.

(4) The cellulose-dissolving bacteria isolated by the writers are morphologically and physiclogically different from the hydrogen and methane ferments of Omelianski.

(5) Some species of cellulose-destroying bacteria, including the thermophiles, lose their power to destroy cellulose very rapidly on artificial media.

(6) Thermophilic cellulose-destroying bacteria are well distributed in nature and are extremely active agents in the destruction of cellulose under favorable temperature conditions.

(7) Filamentous fungi play a much more important rôle in the destruction of cellulose in soils, especially in alkaline soils, than has hitherto been supposed.

(8) The cellulose-destroying molds act differently toward different kinds of cellulose.

(9) The gaseous products attributed to the cellulose ferments by earlier investigators are due to secondary fermentations induced by other organisms.

(10) The principal products formed by some species of cellulose-dissolving bacteria consist of the lower fatty acids; with other species only traces of fatty acids are formed. No aldehydes, ketones, alcohols, or reducing sugars were produced by any of the species examined.

(11) An accurate knowledge of the by-products formed by different species of cellulose-destroying bacteria will aid in a specific classification of these organisms.

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PLATES.

DESCRIPTION OF PLATES.[1]

PLATE I. Colonies and vegetative cells of *Bacterium liquatum* and *Pseudomonas subcretus*. Fig. 1.—*Bacterium liquatum*. Cellulose-agar plate, 15 days at 30° C. Normal size. Fig. 2.—*Bacterium liquatum*. Starch-agar plate,[2] 5 days at 30° C. Normal size. Fig. 3.—*Bacterium liquatum*. Vegetative cells from 24-hour culture on beef agar, aqueous fuchsin stain. Magnification 1,000. Fig. 4.—*Pseudomonas subcretus*. Vegetative cells from 24-hour culture on starch agar, carbol fuchsin stain. Magnification 1,000. Fig. 5.—*Pseudomonas subcretus*. Cellulose-agar plate, 15 days at 30° C. Normal size. Fig. 6.—*Pseudomonas subcretus*. Starch-agar plate, 5 days at 30° C. Normal size.

PLATE II. Colonies and vegetative cells of *Bacterium fimi* and *Bacillus bibulus*. Fig. 1.—*Bacterium fimi*. Cellulose-agar plate, 15 days at 30° C. Normal size. Fig. 2.—*Bacterium fimi*. Starch-agar plate, 5 days at 30° C. Normal size. Fig. 3.—*Bacterium fimi*. Vegetative cells from 24-hour culture on beef agar, carbol fuchsin stain. Magnification 1,000. Fig. 4.—*Bacillus bibulus*. Vegetative cells from 24-hour culture on beef agar, carbol fuchsin stain. Magnification 1,000. Fig. 5.—*Bacillus bibulus*. Cellulose-agar plate, 15 days at 30° C. Normal size. Fig. 6.—*Bacillus bibulus*. Starch-agar plate, 5 days at 30° C. Normal size.

PLATE III. Colonies, spores, vegetative cells, and involution forms of *Bacillus cytaseus*. Fig. 1.—Cellulose-agar plate, 15 days at 30° C. Normal size. Fig. 2.—Starch-agar plate, 5 days at 30° C. Normal size. Fig. 3.—Spores from fermenting filter paper, 9 days at 30° C., aqueous fuchsin stain. Magnification 1,000. Fig. 4.—Vegetative cells from 24-hour culture on potato agar at 30° C., carbol fuchsin stain. Magnification 1,000. Fig. 5.—Involution forms from 48-hour culture on potato agar, carbol fuchsin stain. Magnification 1,000.

PLATE IV. Vegetative cells of *Bacillus bibulus* and *Bacillus cytaseus*, showing flagella. Figs. 1 and 2.—*Bacillus bibulus*. Vegetative cells from 24-hour culture on beef agar, Dr. Hugh Williams's flagella stain. Magnification 1,000. Figs. 3 and 4.—*Bacillus cytaseus*. Vegetative cells from 24-hour culture on potato agar, Dr. Hugh Williams's flagella stain. Magnification 1,000.

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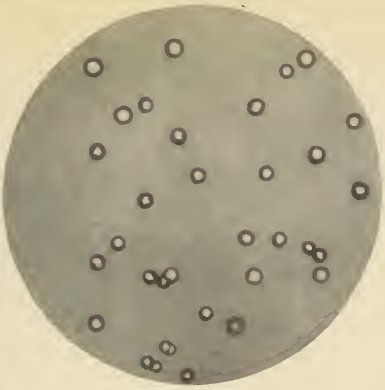
[1] The photomicrographs and photographs here reproduced were made by Mr. F. L. Goll, of the Office of Soil-Bacteriology and Plant-Nutrition Investigations.

[2] A small quantity of 95 per cent alcohol was poured over the surface of all the starch-agar plates to bring out the enzymic zone.



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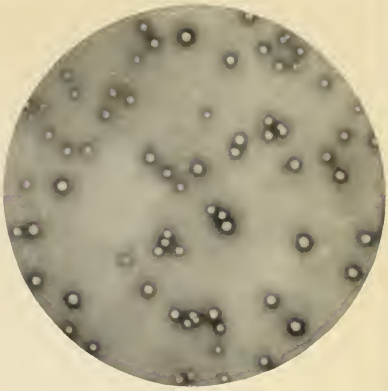

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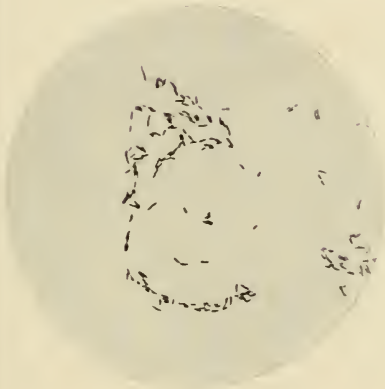

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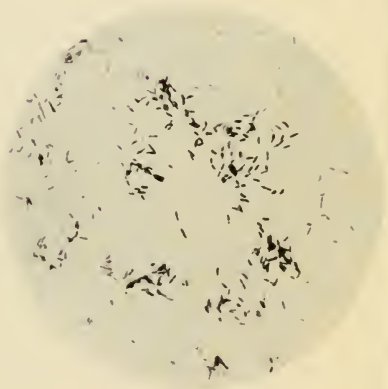

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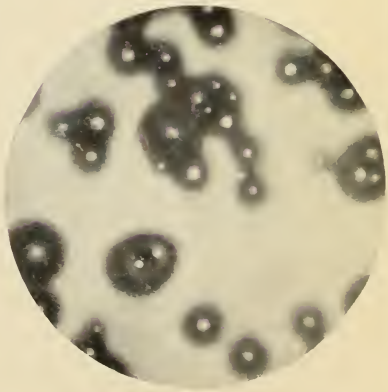

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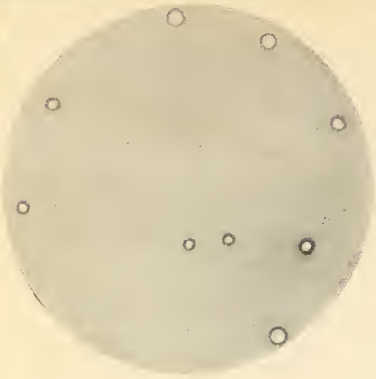
COLONIES AND VEGETATIVE CELLS OF BACTERIUM LIQUATUM AND PSEUDOMONAS SUBCRETUS.



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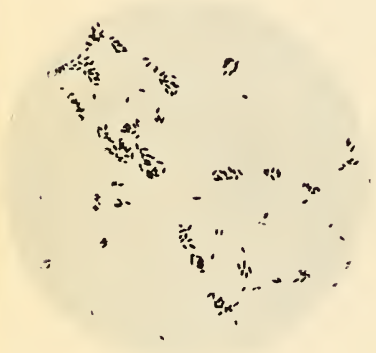

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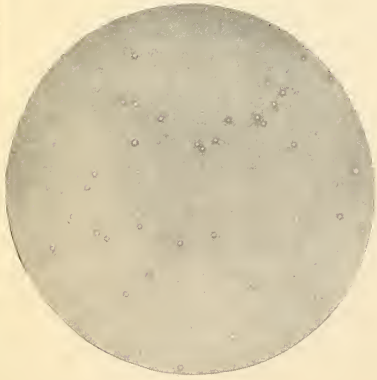

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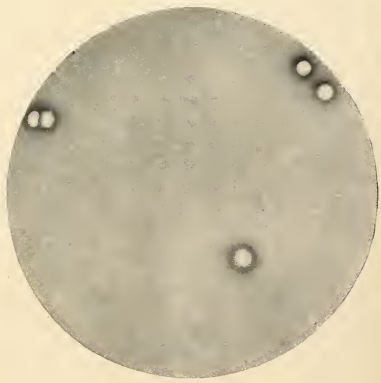

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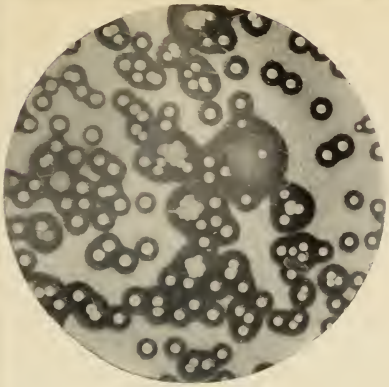
COLONIES AND VEGETATIVE CELLS OF BACTERIUM FIMI AND BACILLUS BIBULUS.



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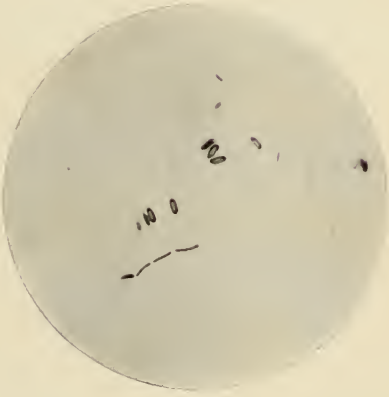

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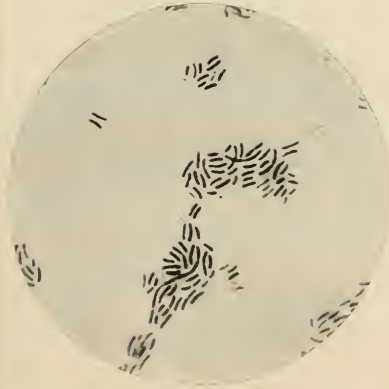

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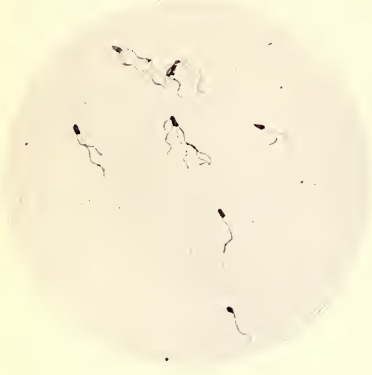
COLONIES, SPORES, VEGETATIVE CELLS AND INVOLUTION FORMS OF BACILLUS CYTASEUS.



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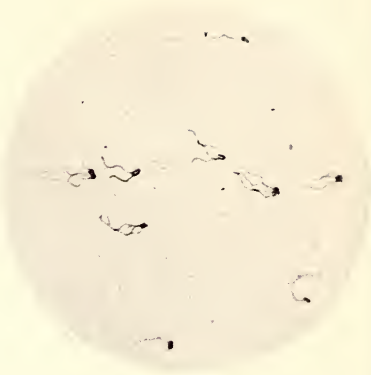

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VEGETATIVE CELLS OF BACILLUS BIBULUS AND BACILLUS CYTASEUS, SHOWING FLAGELLA.